Cell, Vol. 89, 1175–1184, June 27, 1997, Copyright ©1997 by Cell Press

Recruitment of p300/CBP in p53-Dependent Signal Pathways

Maria Laura Avantaggiati,*# Vasily Ogryzko,† Kevin Gardner,* Antonio Giordano,§ Arthur S. Levine,‡ and Kathleen Kelly*‖

*Laboratory of Pathology
National Cancer Institute
†Laboratory of Molecular Growth Regulation
‡Section on DNA Replication, Repair, and Mutagenesis
National Institute of Child Health and Human Development
National Institutes of Health
Bethesda, Maryland 20892
§Sbarro Institute for Cancer Research and Molecular Medicine
Thomas Jefferson University
Philadelphia, Pennsylvania 19107

Summary

The products of the *p53* and *CBP/p300* genes have been individually implicated in control of cell growth and regulation of transcription. p53 is known to act as a positive and negative regulator of gene expression. Here we show that p53, in both wild-type and mutant conformation, forms a specific protein complex with p300. However, in its wild-type but not mutant conformation, p53 inhibits a promoter containing the DNA-binding sequences for the transcription factor AP1, in a p300-dependent manner. p300 stimulates the transcriptional activity of p53 on p53-regulated promoters, and it enhances the responsiveness to a physiological upstream modulator of p53 function, ionizing radiation. A dominant negative form of p300 prevents transcriptional activation by p53, and it counteracts p53-mediated G1 arrest and apoptosis. The data implicate p300 as an important component of p53-signaling, thus providing new insight into the mechanisms of cellular proliferation.

Introduction

p300 and CBP are highly homologous nuclear proteins originally identified for their ability to interact with adenovirus E1a proteins and with the transcription factor CREB, respectively. Both members of this family are regulators of transcription and interact with a variety of cellular as well as viral proteins. Among these are SV40 large T antigen (Tag), transcription factors such as MyoD, c-Jun, JunB, c-Fos, Myb and YY1, nuclear receptors, and basal components of the transcriptional apparatus (Janknecht and Hunter, 1996a, and references therein). In addition, p300/CBP possesses histone acetyltransferase activity resulting either from intrinsic activity and/or from an associated protein, P/CAF (Bannister et al., 1996; Ogryzko et al., 1996; Yang et al., 1996). The acetylation of histones is thought to be involved in destabilization and restructuring of nucleosomes, which is likely a crucial event for accessibility of transcription factors to DNA templates. These studies thus suggested that p300/CBP participates to the transcription process by scaffolding different classes of transcriptional regulators on specific chromatin domains.

A role for p300/CBP in control of cellular growth has been proposed on the basis of the functional behavior of adenovirus E1a and SV40 Tag proteins, which lose the ability to bind to p300/CBP (Moran, 1993; Avantaggiati et al., 1996; Eckner et al., 1996a). Such mutants are defective in the induction of cellular DNA synthesis and, in several instances, of transformation. In addition, we and others have shown that the activity(s) of p300/CBP is required for the activation of muscle-specific genes and for cell cycle arrest during differentiation of muscle cells (Eckner et al., 1996b; Puri et al., 1997). Moreover, mutations or translocations of the *p300*, or of the *CBP*, genes have been described in human tumors (Borrow et al., 1996; Muraoka et al., 1996). p300 mutations in colorectal carcinomas are somatic and coupled to deletion of the second allele of the gene, suggesting that p300 is consequently inactivated. On the basis of this evidence, p300/CBP is envisioned as a negative regulator of cell growth.

The p53 tumor suppressor is also a common target for genetic alteration in human cancers (reviewed in Haffner and Oren, 1995; Ko and Prives, 1996; Levine, 1997). Such genetic lesions disrupt the tumor suppressive function of the protein and, in specific instances, actively contribute to uncontrolled proliferation (Jenkins et al., 1985; Hinds et al., 1989). Wild-type p53 limits cellular proliferation by inducing either a transient G1 arrest or apoptosis, depending on the cellular context. A molecular explanation for the growth-arrest response mediated by p53 relies on its ability to act as a sequence-specific DNA-binding transcription factor. Various downstream target genes of p53 have been identified, such as *p21/WAF*, *MDM2*, *GADD45*, *cyclin G* and *bax*, whose expression products function as regulators of diverse aspects of cell growth (reviewed by Ko and Prives, 1996; Levine, 1997). Several p53 mutations found in human tumors occur in the region encoding the DNA-binding domain and, consequently, mutant p53 proteins lose transactivating activity. Therefore, at least one unifying feature that might account for the different biological effects of mutant p53 consists of their altered ability to modulate gene expression.

In addition to activating transcription, p53 acts as a negative regulator of genes whose promoters do not contain p53-binding sites. Although most studies demonstrating p53-mediated transrepression have utilized transient expression assays, endogenously repressed genes have recently been identified (Miyashita et al., 1994; Murphy et al., 1996). The mechanism by which p53 exerts this inhibition is likely to be through its physical interaction with either basal components of the transcriptional machinery, such as TBP, or other unidentified transcription factors (Ko and Prives, 1996, and references therein). Significantly, the transrepression function of p53 appears to be especially important for the

‖To whom correspondence should be addressed.
#Present address: Department of Microbiology, School of Medicine, State University of New York, Buffalo, New York 14214.

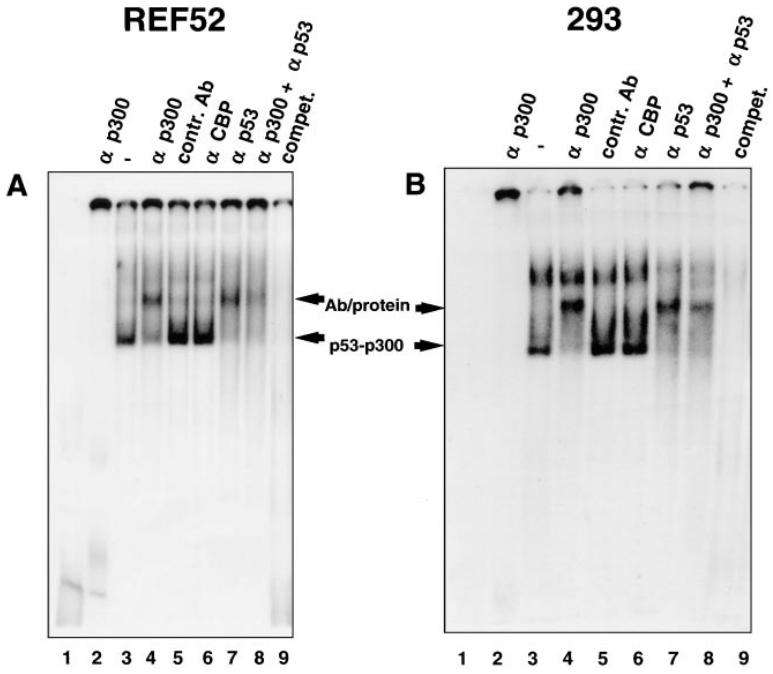

Figure 1. p300/CBP Is Detected in DNA-Bound Complexes together with p53

Binding activity of cellular nuclear extracts derived from a rat embryo-fibroblast cell line, REF52, and from the embryo kidney carcinoma cell line, 293, (lanes 3–9, panels [A] and [B], respectively), to an oligonucleotide carrying three adjacent copies of the p53-DNA-binding site. In both panels, lanes 3 show the binding pattern to the DNA obtained with the extracts alone: a major DNA–protein complex is indicated by the lower arrows (marked p53-p300). For the other lanes, the antibodies indicated at the top of the panels were added to the binding reactions. Incubation with either the anti-53 antibody pAb421 (lanes 7), or the anti-p300 polyclonal (lanes 4) generates a band with lower mobility (marked Ab/protein, upper arrow). In lanes 5 and 6, nuclear cell extracts were incubated with a control preimmune serum and with an anti-CBP specific monoclonal antibody (UBI), respectively. In lanes 8, the anti-p53 and anti-p300 antibodies were added together. Lanes 9 contain the competition with a 10-fold molar excess of unlabeled oligonucleotide. In lanes 2, the anti-p300 polyclonal antibody was incubated with the probe in the absence of the cell extracts.

apoptotic activity of the protein (Levine, 1997), as suggested by the fact that proteins inhibiting p53-mediated apoptosis, such as Bcl2 and the adenovirus E1B, prevent trancriptional repression without affecting transcriptional activation by p53 (Shen and Shenk, 1994). Mutated forms of p53 found in human cancer cells have been shown to lose their transrepression function and can even acquire a stimulatory activity on the same promoters, PCNA and MDR1, for example, that are repressed by wild-type p53 (Chen et al., 1992; Deb et al., 1992).

Because p300/CBP and p53 are both implicated in regulation of transcription and control of cell growth, we sought to investigate the possibility of a functional cross-talk between these proteins. In this study, we show that p53 and p300 form a specific protein complex. We demonstrate that p53-mediated inhibition of AP1-regulated enhancers and transcriptional activation of promoters containing a p53 consensus site are both p300 dependent. Furthermore, a temperature-sensitive p53 mutant (p53 Val135) continues to bind to p300 but, in contrast with the wild-type, stimulates AP1 transcription. A dominant negative form of p300 inhibits p53-dependent transcription, and it counteracts radiation-induced G1-arrest and apoptosis. Our results implicate p300 as an important effector of several of the known activities of p53. Given that p300 appears to be a target for both transcriptional repression and activation by p53, we propose that the modulation of p300 activities at targeted promoters plays a role in the execution of p53-dependent signal pathways.

Results

p300 Interacts with p53

We investigated whether p53 and p300 can be detected in a specific protein complex bound to the DNA. Electrophoretic mobility gel assays (EMSA) were performed using an oligonucleotide containing three copies of the p53 consensus site derived from the promoter of the *p21/WAF1* gene. Nuclear extracts of REF52 and 293 cells (Figures 1A and 1B, respectively) gave rise to a major band (lanes 3, both panels) that was competed by an excess of cold oligonucleotide and supershifted by the anti-p53 antibody pAb421 (lanes 7 and 9, respectively). The mobility of this p53-containing complex was significantly retarded by a polyclonal antibody directed against both CBP and p300, but not by a preimmune rabbit antiserum (lanes 4 and 5) nor by a monoclonal antibody directed against CBP (lanes 6). Incubation of cell extracts with the anti-p53 and anti-p300 antibodies together resulted in a further diffuse supershifted band (lanes 8). Hence p300/CBP is contained in a specific DNA-bound protein complex together with p53.

To confirm the ability of p300 to bind to p53, immunoprecipitations followed by Western blots were performed. CV1 cells were transfected with a plasmid encoding wild-type p53, CMVp53, and 48 hr later, cell extracts derived from the transfected cells were subjected to immunoprecipitation with either a monoclonal anti-p53 antibody, pAb421, or with the polyclonal anti-p300 antiserum (Figure 2A, lanes 2 and 3, respectively). Western blot analysis of these precipitates revealed the presence of a discrete fraction of p300 associated with p53. In contrast, we could not detect CBP in association with p53. Whether this is due to a relative insensitivity of the immunoprecipitation assays or to an exquisite specificity of p53 for p300, is unclear.

We next investigated whether p53 requires its native conformation to bind p300 by utilizing a temperature-sensitive p53 mutant that substitutes valine for alanine in position 135 (p53 Val135) (Michalovitz et al., 1990). An embryonic fibroblast cell line derived from homozygous null mice for the *p53* gene (MEF) was transfected with

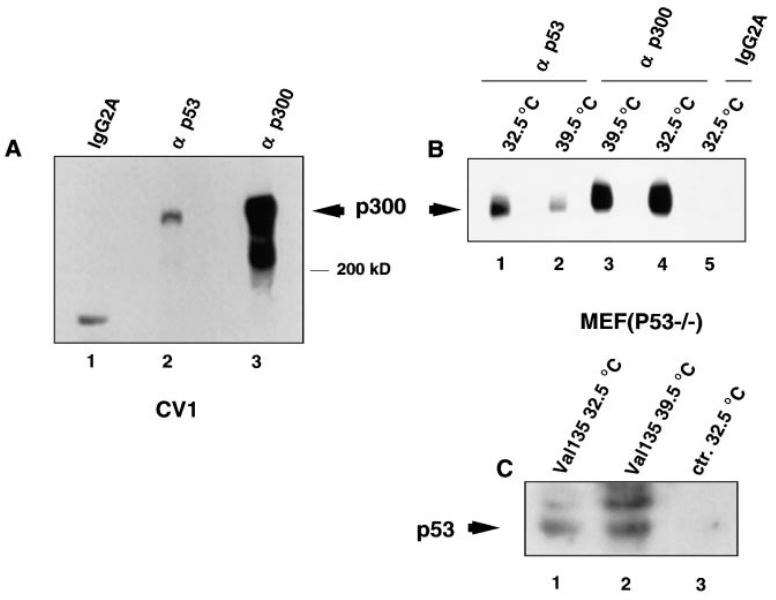

Figure 2. p300 Interacts with Wild-Type and Mutant p53

(A) Cell extracts derived from CV1 cells transfected with 10 μg of the p53-expressing vector (CMVp53) were immunoprecipitated with a control, null, IgG2A monoclonal antibody (lane 1), with the anti-p53 antibody pAb421, (lane 2), or with the anti-p300 polyclonal antiserum (lane 3). After electrophoresis and electroblotting, the membranes containing immobilized immuno-complexes were subjected to Western blot by using a mixture of three p300 monoclonal antibodies (RW128, RW105, RW109, UBI).

(B) Mouse embryo fibroblasts derived from double *p53* null gene (*MEFp53*$^{-/-}$) mice were transfected with a CMV-driven plasmid encoding the temperature-sensitive p53 mutant, p53Val135. Cells were incubated at the permissive (32.5°C, lanes 1 and 4) or nonpermissive (39.5°C, lanes 2 and 3) temperature for 36 hr after transfection. Cell extracts derived from the transfected cells were mixed with similarly prepared extracts of mock-transfected cells, immunoprecipitated with either anti-p53 (lanes 1 and 2) or anti-p300 antibodies (lanes 3 and 4), and assayed for the presence of p300 in a Western blot, as described in (A). Lane 5 contains the immunoprecipitation with the control, null, IgG2A antibody from the mock-transfected cells incubated at 32.5°C.

(C) 100 μg of total extracts derived from the transfection in (B) were probed in a Western blot with the pAb421 to monitor the expression of wild-type and mutant p53. Lanes 1 and 2 contain extracts from cells transfected with p53Val135 and incubated at the permissive or nonpermissive temperature, respectively. Cell extracts derived from the nontransfected cells were loaded in lane 3. In all panels, the position of p300 and p53 is marked by the arrows.

a vector encoding p53 Val135. Cell extracts were immunoprecipitated with the anti-p53 antibody pAb421, which recognizes both wild-type and mutant conformation, or with the anti-p300 antiserum (Figure 2B). To monitor the expression of p53, an anti-p53-specific Western blot was performed (Figure 2C). p300 was detected in the anti-p53 immunoprecipitations derived from cells incubated at the permissive (32.5°C) and, to a lesser extent, in the cells incubated at the nonpermissive (39.5°C) temperature (Figure 2B, lanes 1 and 2, respectively), indicating that both wild-type and mutant p53 are capable of interacting with p300. Moreover, in keeping with the results shown above, it appears that complex formation between p300 and p53 is conserved among different cell lines, suggesting the involvement of these proteins in common function(s).

To identify the region of p300 that interacts with p53, a series of p300 deletion mutants translated in vitro in presence of *35*S-cysteine were individually mixed with cellular extracts containing endogenous p53 and immunoprecipitated with the anti-p53 antibody. The nature of these mutations is illustrated in Figure 3A. The region of p300 extending from residues 1514 to 1922, p300(1514–1922), exhibited the highest binding activity for p53, while neither a large internal deletion, p300(D242–1737), nor a p300 fragment containing only the amino-terminal portion were capable of generating detectable interactions (Figure 3B). These results were confirmed with a second approach by using baculovirus-expressed p300 proteins (data not shown).

Therefore, p53 binds to the C-terminal domain of p300 between amino acids 1514–1737, which encompasses a region upstream and partially included in the third cysteine/histidine-rich domain.

p300 Is an Important Component of p53-Dependent Transcription

Based on the results shown above, and because p300 and p53 are both transcriptional regulators, we investigated the effects of p300 on p53-dependent transcription. The p300-expressing vector (CMVp300), alone or in combination with a vector encoding p53 (CMVp53), was cotransfected into MEF(p53$^{-/-}$) cells together with a reporter containing a synthetic p53 binding site placed upstream of the luciferase gene (PG13). The expression of p300 in MEF cells had no significant effect on the levels of activation of the reporter in the absence of cotransfected wild-type p53 (Figure 4A). Transfection of the p53-expressing vector produced a marked stimulation of transcription, as expected. A significant stimulation of the reporter above the levels observed with p53 alone was specifically induced by coexpression of full-length p300. This stimulatory effect was not observed following exogenous expression of p300(D242–1737), which lacks the p53 binding site. Therefore, the transcriptional activity of p53 is enhanced by p300.

The structural and functional properties of p300 are consistent with the possibility that it acts as a transcriptional adapter molecule that scaffolds transcription factors and basal components of the transcriptional machinery at targeted promoters. Disruption of the activity of p300 is thus expected to perturb transcriptional activation. Thus, the effects of the p300(1514–1922), which contains the minimal regions of p53 binding but not other regulatory domains, were examined. We anticipated that this molecule might prevent the activation of p53-regulated promoters by interfering with complex formation between endogenous p53 and p300. The expression of p300(1514–1922) inhibited the activation

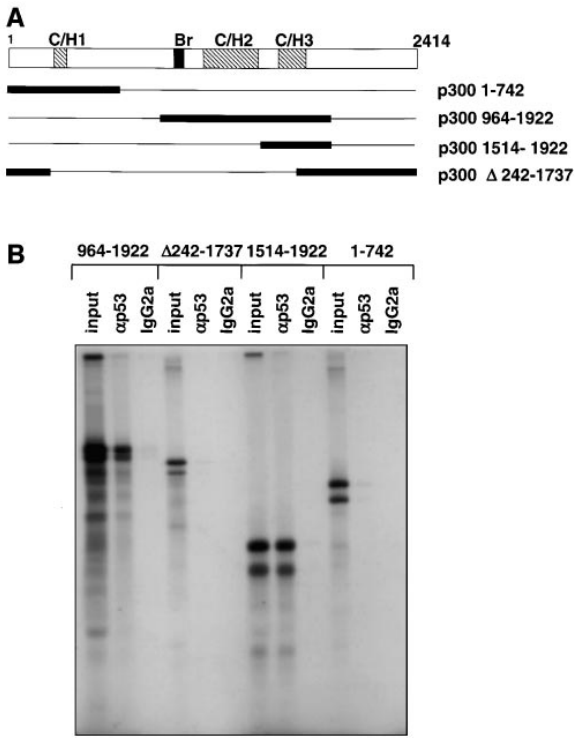

Figure 3. Regions of Interaction between p53 and p300

(A) Schematic diagram of the four p300 deletion used. Thick and thin lines indicate included and excluded sequences, respectively. The bromodomain (marked as Br by shaded box) and the three cysteine/histidine-rich domains (C/H1, C/H2 and C/H3, striped boxes) are indicated.

(B) The various p300 proteins were translated in vitro by using a coupled transcription/translation reticulocyte-based system (Promega) in the presence of ^{35}S-cysteine. Five microliters of each translation reaction was run on an 8% gel and the efficiency of translation was monitored by autoradiography of the gel (not shown). The quantities of translated proteins were normalized accordingly to expose p53 to similar amounts of p300. Translated proteins were mixed with 1 mg of cell extracts derived from Cos7 cells, preincubated on ice for 30′, and immunoprecipitated with a control IgG2A or with p53 specific antibodies as indicated at the top of the panels.

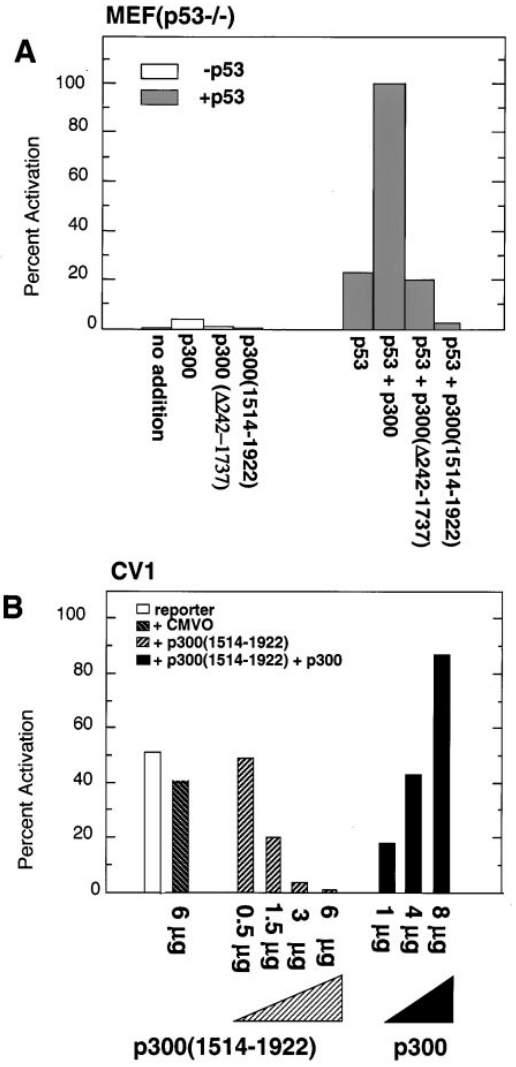

Figure 4. p300 Enhances p53-Directed Transcription

A luciferase reporter containing 13 copies of a synthetic p53 consensus site derived from the promoter of p21/WAF1 (PG13) was used in these assays.

(A) MEF cells (*p53*$^{-/-}$) were cotransfected with 0.5 μg of the p53 reporter and 1.5 μg of the plasmids expressing full-length p300 (CMVp300); or p300(1514–1922), containing the regions required for the interaction with p53; or p300(Δ242–1737), which does not bind to p53. The left and the right of the panel (open bars and striped bars, respectively) show the levels of luciferase activity obtained in MEF cells cotransfected with the various p300 fragments in the absence or presence of 1.5 μg of cotransfected p53, respectively.

(B) p300(1514–1922) inhibits p53-dependent transcription in a dose-dependent manner. CV1 cells were transfected with 0.5 μg of the p53 reporter alone (open bar) or with increasing concentrations of p300(1514–1922) (0.5, 1.5, 3, and 6 μg, light striped bars), with 6 μg of the control plasmid CMV0 (dark striped bar). To explore whether the inhibition of p53-mediated transcription produced by p300 (1514–1922) can be restored by full-length p300, maximally inhibitory concentrations (6 μg) of the CMVp53 plasmid were cotransfected together with increasing amounts (1, 4, and 8 μg, black bars) of CMVp300. Cells were transfected with lipofectamine. The total DNA content was equalized in each sample with the backbone plasmid, CMV0, in (A), and with the pUC19 plasmid in (B). Samples were assayed for luciferase activity 36–48 hr after transfection, and equal amount of cell lysates were used for detection. The highest value was arbitrarily set as 100 and all other consequently adjusted.

driven by p53 in cells lacking endogenous p53 (Figure 4A). Moreover a dose-dependent inhibition of the p53 reporter was observed in CV1 cells that constitutively produce p53 (Figure 4B). Significantly, this inhibition was reversed by increasing concentrations of the plasmid encoding full-length p300, suggesting that p300(1514–1922) likely acts by competing with endogenous p300 for p53 interaction. No differences in the expression levels and in the subcellular localization of p53 were observed as a consequence of overexpression of the p300(1514–1922) (see Figure 7). Thus, p300 appears to be an important component of p53-directed transcription.

Recent evidence indicates that p53 is crucially involved in a signal pathway that is induced by ionizing radiation (IR) and, through the activation of p53-responsive genes, leads to arrest of the cell cycle at the G1/S and G2/M checkpoints (Ko and Prives, 1996; Levine, 1997; and references therein). To establish whether p300

is involved in IR responsiveness, we examined the effects of p300 expression on p53-dependent transcription following IR treatment of the human breast carcinoma cells, MCF7. The transcriptional activity of p53 was enhanced by signals initiated by IR, as indicated by a time-dependent increase in the activity of the reporter in cells transfected with p53 alone (Figure 5A). This could be related to changes in the expression levels of p53 induced by irradiation (see Figure 5B) and/or to qualitative modifications that enhance the intrinsic activity of p53. Similarly, p300-mediated stimulation of p53 transcription was significantly higher in irradiated cells. This suggests that p53 and p300 act in concert to modulate intracellular signals activated by DNA damage.

Wild-Type and Mutant p53 Perturb p300-Mediated Activation of the TRE

Wild-type p53 is known to inhibit the expression of several genes whose promoters do not contain a p53-binding site. This inhibition has been attributed to the interaction and functional sequestration of several components of the transcriptional apparatus (Ko and Prives, 1996). Thus, we sought to examine the effects of p53 on a promoter containing the DNA consensus site for the transcription factor AP1 (TRE), whose activation is p300-dependent (Arias et al., 1994). Transfection of the plasmid encoding the temperature-sensitive mutant p53 (p53 Val135) resulted in a strong inhibition of the TRE reporter after incubation of the cells at the permissive temperature (Figure 6A). Incubation at the restrictive temperature that induces the mutant conformation produced a small but reproducible stimulation of the TRE. AP1-dependent transcription was enhanced upon expression of full-length p300 and, to a lesser extent, of p300(Δ242–1737), which contains a carboxyl-terminal activation domain (Yuan et al., 1996) along with the binding site for c-Jun (Lee et al., 1996), one of the main components of the AP1 class of transcription factors. Transfection of the vector encoding wild-type p53 resulted in a dose-dependent inhibition of the reporter activity (data not shown), and high concentrations of the p53-encoding plasmid almost completely silenced TRE transcription (Figure 6B). Strikingly, p53 prevented the activation of the reporter driven by full-length p300 but not by p300(Δ242–1737), which lacks the region required for the interaction with p53. These results suggest that the association between p53 and p300 might, at least partially, account for p53-induced inhibition of the TRE.

One possibility suggested by the above data is that the formation of p53-p300 complexes reduces the amount of p300 available for transcriptional activation. In such a case, overexpression of p300 is expected to overcome the inhibition. As shown in Figure 6C, transfection of the vector encoding p300 prevented p53-imposed repression of the TRE reporter in a dose-dependent manner. On average, more than half of the original enhancer activity was restored in these assays. To rule out the possibility that the interaction of p53 with p300 might structurally prevent other components of AP1 from binding to the DNA, gel retardation experiments were performed. As shown in Figure 6D, neither wild-type nor mutant p53 modified the total amounts of the DNA-bound complexes or their composition, since Jun and Fos appear to be the main components of AP1 in the presence or absence of overexpressed p53.

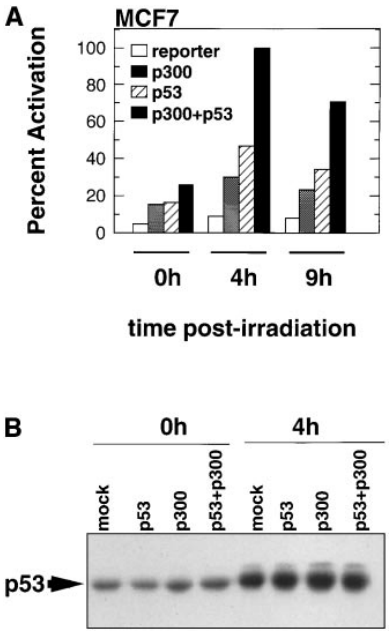

Figure 5. Effects of p300 on p53-Dependent Transcription during IR Treatment of MCF7 Cells

(A) Human breast carcinoma cells (MCF7) were transfected with 0.5 μg of the p53 reporter and 1 μg of the vector encoding full-length p300 (CMV-p300) alone or in combination with 1 μg of the p53-expressing plasmid (CMV-p53), by using a calcium phosphate method (Pharmacia). The levels of DNA were equalized in all samples with the backbone vector, CMV0. Eighteen to 24 hr after transfection, cells were exposed to a total of 6.3 Gy, ^{137}Cs source delivering gamma-rays at a dose rate of 3.46 Gy/min. Equal amounts of cell lysates were assayed for luciferase activity at the indicated times thereafter (0, 4, 9 hr, respectively).

(B) Total cellular extracts derived from nonirradiated (0 hr) or irradiated cells (4 hr) and transfected as indicated at the top of the panel were probed in a Western blot with an ascites-purified anti-p53 antibody, pAb421.

Effects of Dominant Negative p300 on p53-Mediated Apoptosis and G1 Arrest

p53 is known to respond to multiple signals of cellular damage by inducing either a transient growth arrest or apoptosis (reviewed by Ko and Prives, 1996; Bates and Vousden, 1996; Levine, 1997). Our findings have implicated p300 as a target of p53 and we have identified a p300 fragment, p300(1514–1922), that interferes with the activity(s) of endogenous proteins. Thus, to establish the role of p300 in p53-signaling we examined the effects of p300(1514–1922) on cells treated with either ionizing radiation or anticancer agents, both of which are essential upstream modulators of p53.

First, the effects of p300(1514–1922) on p53-directed

These sets of experiments were performed multiple times in different cell lines, and the results shown represent the average of three independent experiments.

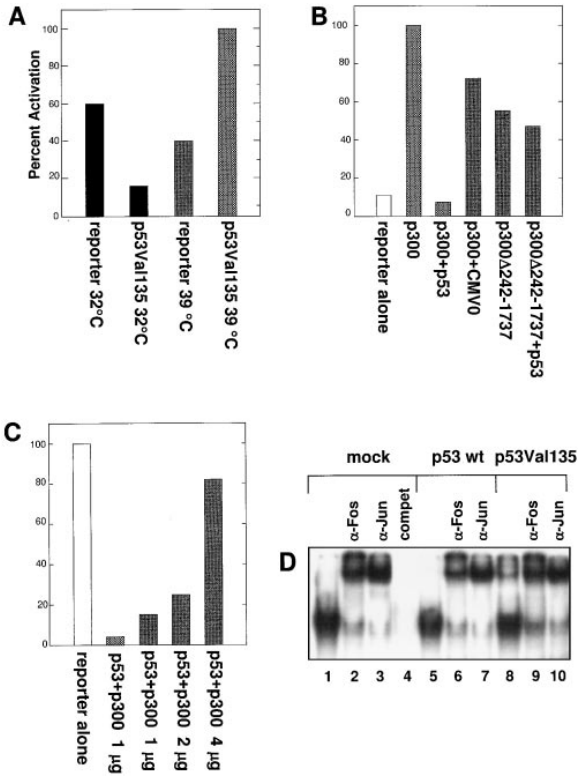

Figure 6. Inhibition of TRE-Mediated Transcription by p53 and Its Reversion by p300

A luciferase reporter plasmid driven by the phorbolester-responsive element (TRE), which contains four copies of the DNA binding sequences for the transcription factor AP1, was used in these assays.

(A) Effects of p53 wild-type and mutant on the TRE. Two micrograms of CMVp53Val135 encoding the temperature-sensitive p53 mutant (p53 Val135) were cotransfected in CV1 cells together with 1 μg of the reporter. Transfection was carried out by using lipofectamine. Cells were incubated at 32.5°C (black bars) or 39.5°C (gray bars) and luciferase levels were determined 24–36 hr later.

(B) p53 inhibits TRE-dependent transcription and its activation by full-length p300 but not by p300 molecules lacking the p53 binding site, p300(Δ242–1737). CV1 cells were cotransfected with 1 μg of the reporter and 2 μg of the plasmids expressing full-length p300, or the p300(Δ242–1737), or the control plasmid CMV0, alone or in combination with 2 μg CMVp53 vector, as indicated in the panel.

(C) Dose-dependent relief of the p53-imposed repression of the TRE by p300. Two micrograms of the CMVp53 plasmid were cotransfected together with increasing concentrations (1, 2, and 4 μg, respectively) of the p300 expressing vector as indicated at the bottom of the panel.

(D) The expression of wild-type p53 and of the mutant p53 Val135 does not modify the composition of the TRE-bound complexes. HeLa cells were mock transfected (lanes 1 to 4) or transfected with either 10 μg of the CMVp53 (lanes 5 to 7) or 10 μg of CMVp53 Val135 (lanes 8 to 10), together with 5 μg of the pHook vector (Invitrogen) encoding a cell surface antigen to select transfected cells. Cells were incubated at 37°C (lanes 1 to 7) or 39.5°C (lanes 8 to 9), and 48 hr later transfected cells were isolated by using a magnetic sorting procedure according to the manufacturer's instruction. Nuclear cell extracts were prepared as described in the Experimental Procedures. EMSAs were performed by using a labeled oligonucleotide containing a single copy of the DNA-binding site for AP1 (Promega). Extracts were incubated with the probe alone (lanes 1, 5, and 8), with an anti-Jun (lane 2, 6, and 9), or anti-Fos (lanes 3, 7, and 10) antibodies, respectively. Lane 4 contains the competition with a 10-fold excess of unlabeled oligonucleotide on nuclear extracts of mock-transfected cells.

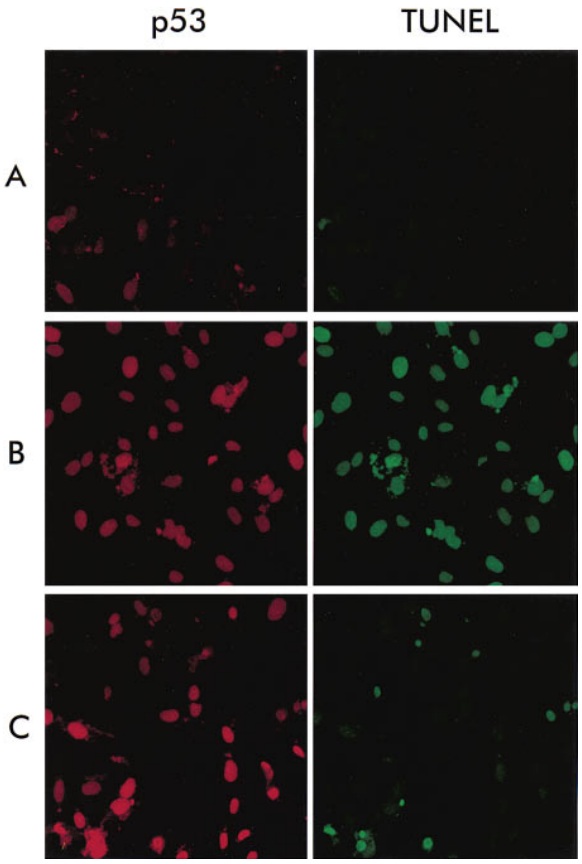

Figure 7. Effects of p300(1514–1922) on p53-Mediated Apoptosis

SAOS-2 cells were transfected with 1/20 (v/v) of a transfection reaction containing 30 μl of lipofectamine (GIBCO–BRL) and: 21 μg of the control, backbone plasmid CMV0 (A); 7 μg of the p53 expressing vector (CMVp53) and 14 μg of the CMV0 (B); 7 μg of the CMVp53 plasmid and 14 μg of the p300(1514–1922) (C). Twenty-four hours after transfection, cells were exposed to doxorubicin (5 μg/ml) and reincubated for an additional 12 hr. Double staining for p53-expression (rodhamine, left panels) and apoptosis (fluorescein, right panels) was performed as described in the Experimental Procedures.

killing of neoplastic cells in response to the anticancer drug doxorubicin were assessed. SAOS-2 cells, which do not express endogenous p53, were transfected either with the control vector CMV0, or with the vector encoding p53 (CMVp53) alone or in combination with the p300(1514–1922). Twenty-four hours after transfection cells were treated with doxorubicin and apoptotic features evaluated 12 hr later. The cells were double-stained for p53 expression and for DNA breaks, characteristic of apoptotic induction (TUNEL). The results are shown in Figure 7, and quantified in Table 1. In the absence of p53 expression, less than 20% of SAOS cells treated with doxorubicin were positive for TUNEL staining (Figure 7A). Approximately 95% of cells transfected with p53 alone (Figure 7B) and treated with doxorubicin demonstrated clear apoptotic features, evaluated by either the intensity of the staining or morphology of the nuclei, indicating that in this cell line and under the conditions that we employed, apoptosis occurs through 53-dependent pathways. Coexpression of p53 and

Table 1. Inhibition of Apoptosis by the p300 (1514–1922)

Vector	Apoptotic Cells (%)
CMVO	14
CMVp53 + CMV0	95
CMVp53 + p300 (1514–1922)	43

Fluorescein-TUNEL assay was performed as described in Figure 7, and the results were quantified by counting the cells of 14 nonadjacent fields of samples derived from a triplicate experiment. The results express percentage values of the number of apoptotic cells.

Table 2. p300 1514–1912 Perturbs IR-Induced Cell-Cycle Arrest

Vector	IR	G1 (%)	S (%)	G2/M (%)
Random phase cells				
CMVO	−	65.4	23.5	9
CMVO	+	70.3	1.3	22.5
p300(1514–1912)	+	74.7	4.2	15.4
Cells treated with nocodazole				
CMVO	−	70	11	15.7
CMVO	+	72	1.3	22.1
p300(1514–1912)	+	63.8	5.8	23.6

MCF7 cells were cotransfected with 15 μg of either the control plasmid CMV0 or with p300(1514–1922) and with 7 μg of a vector encoding the cell surface receptor for IL2 (CMV-IL2R). Nontreated (random phase) and nocodazole treated (15 μg/ml) cells are shown. Nocodazole was added 4 hr after IR. Cells were harvested 24 hr after IR, and they were labeled during the final 30 min of culture with BrdUrd. Magnetic affinity cell sorting was performed as described in the Experimental Procedures. For flow cytometry, nuclei from transfected cells were fixed with ethanol and stained with FITC-conjugated anti-BrdUrd (Becton-Dickinson) and propidium iodide according to the manufacturer's protocol. Stained nuclei were analyzed on FACScan (Becton-Dickinson) using LysisII software. The number of cells in each sample analyzed was approximately 5×10^3. The experiments were performed 4 times and 2 times for random phase or nocodazole-treated cells, respectively, with results similar to those shown.

p300(1514–1922) resulted in a significant reduction of the cells in late or early apoptotic stages (Figure 7C). The percentage of apoptotic cells in the presence of the p300(1514–1922) was approximately 43%, twice the levels seen in the p53 (−) background, and about half the levels obtained by expression of the p53 alone (Table 1), thus indicating that p300(1514–1922) induces a significant inhibition of p53-mediated apoptosis.

In a further set of experiments, we investigated whether p300(1514–1922) could perturb p53-mediated G1 arrest following IR. The human breast carcinoma cell line MCF7 was utilized because it responds to IR treatment with cell-cycle arrest at the G1 and G2 checkpoints in a p53-dependent manner (Fan et al., 1995). The vector encoding p300(Δ1514–1922) or the control vector CMV0 were transfected into MCF7 cells together with a plasmid encoding the IL2 receptor, which served as a selection marker to isolate transfected cells (Giordano et al., 1991). Sixteen to 18 hr after irradiation, cells were processed for cell cycle analysis. The effect of p300(1514–1922) was examined first in random-phase exponentially growing cell cultures. Treatment of control cells with IR induced cell cycle arrest in G1 and G2 and a significant reduction in the cells progressing through S phase (Table 2). This is consistent with p53 acting at the G1/S and G2/M checkpoints (Ko and Prives, 1996; and references therein). Expression of p300(1514–1922) partially inhibited the radiation-induced G2 arrest and increased the percentage of the cells in S phase. Despite an increase in the percentage of cells in S phase, which are drawn from the pool of the cells in G1, the percentage of cells in G1 also was found to be increased, likely as a consequence of the cells released from the G2/M block. To evaluate more clearly the effects of p300 on G1/S transition, similar experiments were next performed in a cell population treated with nocodazole to inhibit transit from G2 to M. In the presence of nocodazole, p300(1514–1922) reduced the fraction of the cells in G1 below that of nonirradiated cells and again increased the fraction of cells in S phase. The data thus support the conclusion that the p300(1514–1922) inhibits p53-dependent signal pathways through dominant negative activity(s) for endogenous p300.

Discussion

The results of this study establish a fundamental role for p300 in the biological activities of p53. The physical association of p53 and p300 was determined by their coprecipitation as soluble complexes or by the ability of an antibody directed against p300 to supershift p53 complexes bound to DNA containing p53-binding consensus sites (Figures 1 and 2). Remarkably, p53-p300 complexes were detected in a variety of cell lines, including human, rat, and mouse cells, thus indicating that this interaction is conserved among different species. Previous reports have shown that complexes between p300/CBP, p53 and the viral protein SV40 large T antigen (Tag) are detected in cells constitutively expressing the early products of SV40 virus (Eckner et al., 1996b; Lill et al., 1997). In keeping with this observation and given the well-known cell cycle and cell-growth regulatory activity of SV40 Tag, it seems likely that p53-p300 complexes might operate in diverse aspects of cellular proliferation. Results presented in this study are consistent with this view. p53 has a well-documented activity as a transcriptional activator, which is clearly required for its cell-cycle arrest function. As shown in Figures 4 and 5, p300 acts cooperatively with wild-type p53 to stimulate transcription from a p53-dependent promoter. A small p300 fragment (p300 1514–1922) containing the region required for the interaction with p53 prevents the activation of a p53-regulated promoter, implying that p300 is an important component of p53-directed transcription. Strikingly, p300 expression significantly increases the responsiveness to a physiological upstream regulator of the action of p53, such as ionizing radiation. Consistent with its ability to interfere with the activity of endogenous p300, p300(1514–1922) inhibited irradiation-induced G1 arrest. For p53, as it is true with a variety of transcription factors, p300 plays a stimulatory role in transactivation. Therefore, in the context of p53-mediated growth suppression, p300 can be viewed as a negative regulator of cell growth.

The activation of several promoter/enhancer elements that also function in a p300-dependent manner, such as the AP1 and c-Fos promoters (Arias et al., 1994), has been correlated to promotion of G1–S transition, cellular

proliferation, and/or transformation (reviewed by Angel and Karin, 1991). Thus, while recruiting p300 on certain promoters, p53 could also inhibit selected transactivating functions of p300 on others. In this study, we have shown that wild-type p53 inhibits p300-mediated activation of a promoter containing the DNA-binding sites for the transcription factor AP1 (TRE) (Figure 6). This effect is at least partially dependent on the formation of p53-p300 complexes since p53 failed to prevent the stimulatory effect of a p300 fragment missing the region required for the association. The identification of p300 as an important component of p53-mediated transcription, together with the notion that the concentration of p300 is rate limiting in the cells (Kamei et al., 1996), suggests a mechanism by which p53 might inhibit AP1 activation based on the requirement for p300 by both classes of transcription factors. The observation that increased levels of p300 overcome p53-mediated inhibition of the TRE strongly supports this interpretation (Figure 6). Non-mutually-exclusive explanations, however, include post-translational modifications of the proteins and/or change in the composition of the complexes that might act to redirect p300 activities from AP1 to p53-regulated promoters. In this regard, it is intriguing that p53 binds to p300 in a region that is required for the intrinsic histone acetyl transferase activity (Ogryzko et al., 1996), but it is distinct from the binding domain for c-Jun (Lee et al., 1996), P/CAF (Yang et al., 1996) and TBP (Yuan et al., 1996), all of which are presumably important modulators of the activity of this protein on certain promoters. Therefore, p53 might function directly, i.e., through protein–protein interactions, by sequestering p300 itself and perhaps other p300-associated factors. As shown here for the p53 Val135 mutation (Figure 6) and elsewhere for others, mutated forms of p53 lose the transcriptional repression activity, thus emphasizing the importance of this function for the integrity of p53-dependent pathways. The finding that p53 Val135 is capable of interacting with p300 (Figure 2), although to a lesser extent, might indicate that the inhibition of AP1-directed transcription requires a native conformation for the interaction with proteins other than p300 (which may themselves be associated with p300) yet is important for transcriptional regulation.

What role does p300 play in p53-directed killing of the cells? Our results imply that the activity(s) of p300 is required for this effect, since a dominant negative form of this protein partially rescued doxorubicin-mediated apoptosis. Recent investigations have shown that the expression of viral or cellular oncogenes, *bcl-2* and *E1B-19K* for example, reverses p53-mediated inhibition of endogenous genes and inhibits apoptosis (Shen and Shenk, 1994; Murphy et al., 1996). Moreover, certain p53 deletion mutants that lack the transactivating function induce apoptosis in several cell lines but not in others (Haupt et al., 1995). Thus, in addition to transactivation-dependent modes of apoptosis, p53 might use alternative pathways, such as the downregulation of genes critical for survival or direct signaling through protein–protein interactions (reviewed by Levine, 1997). Data presented here have implicated p300 in both the transrepressing and transactivating activity of p53 suggesting that the imbalance of p300 activity(s) at targeted promoters might play a role in the execution of p53-mediated apoptosis. Moreover, although the evidence implicating p300 as an integrator of transcriptional responses is overwhelming, it is likely that p300-activities other than transcriptional regulation do exist, as indicated by the ability of this protein to associate with cell-cycle regulated cyclins and kinases (Yaciuk and Moran, 1991; Perkins et. al., 1996; Janknecht and Hunter, 1996b, and references therein). Thus, the establishment of p300 as an effector of p53-dependent pathways then suggests an important point of potential cross-talk between p53 and different classes of regulatory proteins in the cell, an especially relevant consideration given the complexity of the downstream events mediated by p53. Because p300 is a multifunctional molecule, it will be very informative to correlate its mutations (either naturally occurring or engineered) with phenotypic readouts. The role of p300 in p53-dependent pathways alerts one to the possibility that mutations of the *p300* gene may be selected to downregulate or eliminate p53 activity(s) in certain situations such as oncogenesis.

Experimental Procedures

Cells, Plasmids, and Antibodies

Monolayers of African green monkey kidney cells, CV1, 293, REF52, SAOS-2, and MEF (*p53*$^{-/-}$) cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). The anti-p300 polyclonal antiserum has been described elsewhere (Avantaggiati et al., 1996). The anti-p300 and anti-CBP monoclonal antibodies were purchased from UBI. The anti-p53 specific antibody, pAb421, employed for immunoprecipitation, is an IgG2A mouse monoclonal antibody purchased from Oncogene Science. The pAb421 used in the gel retardation assays is an ascities-purified antibody provided by T. Melendy. The CMV-p300 plasmid was provided by R. Eckner and D. Livingston; the CMVp53 Val135 was a gift from M. Oren.

Transfections and Reporter Assays

Transfections were performed by using either lipofectamine (GIBCO–BRL) or a calcium phosphate-based method (Pharmacia). Early-passage cell cultures were employed for both reporter assays and immunoprecipitation experiments. For transactivation assays, cells were plated at 20%–40% confluence 12–18 hr prior to transfection. All expressing vectors employed in this study were driven by the CMV promoter. Unless otherwise indicated, transfections were carried out by using a ratio between reporter and activators of 1:2. Usually, 0.5–1 μg of reporter plasmid and 1–2 μg of activator were transfected in the combinations indicated in the figure legends. When necessary the total content of transfected DNA was equalized with either the control backbone plasmid, CMV0, or with pUC19. Cells were exposed to lipofectamine or to calcium phosphate for about 12 hr, washed twice with PBS, and reefed with complete medium. Equal amounts of cell lysates were employed for detection of luciferase activity (Promega).

Immunoprecipitations and Western Blot

One to 3 mg of total cell extracts was prepared from either transfected or untransfected cells by incubating the cells in buffer A (20 mM $NaPO_4$ [pH 7.8], 240 mM NaCl, 0.1% NP40, 5 mM EDTA, 1 mM DTT) supplemented with freshly prepared protease and phosphatase inhibitors (10 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonylfluoride, and leupeptin, aprotinin, and pepstatin at 10 μg/ml each). After incubation (10′, 4°C), extracts were centrifuged at 12,000 rpm, and the supernatants were collected. Pellets were resuspended in 2 vol of buffer A, vortexed, and incubated on ice for an additional 10 min. Extracts from these two extractions were combined and precleared twice with an excess of Protein A. Typically, 1/5 of the total extract was immunoprecipitated with the anti-p300 antiserum and the remaining with the anti-p53

antibody. The volumes of the reactions were below 1 ml, and the antibodies dilutions were 1:100 for the anti-p300 antiserum and 1/10 (v/v) for the pAb421. Immunoprecipitations were carried out at 4°C for 45–90 min, precipitates were washed 3–6 times in lysis buffer, eluted in 2× SDS sample buffer, loaded on 7.5% SDS–PAGE gels (30:0.7 Acryl/Bis), and run overnight. After transfer at 65 V for 4.5 hr at room temperature, membranes were incubated in blocking solution (10% Horse Serum, 0.1% Tween 20, in 1× PBS) for 6 hr and overnight with anti-p300 antibody (UBI). Immunocomplexes were detected by using a chemiluminescence-based system (Amersham) according to the manufacturer's instructions.

Gel Retardation Assays

The oligonucleotide probe containing the AP1 site was purchased from Promega. The p21/WAF oligonucleotide contained three copies of the p53 binding site (GAACATGTCCCAACATGTTGGAACATG TCCCAACATGTTGGAACATGTCCCAACATGTTG) derived from the promoter of p21/WAF. Nuclear cell extracts were prepared from a minimum of 10^7 cells. Cells were resuspended in 5 vol of buffer A (10 mM HEPES [pH 7.5], 1 mM KCl, 1.5 mM $MgCl_2$, 4 mM BME, 0.5 mM PMSF, 10 mg/ml of aprotinin, leupeptin, and pepstatin), incubated on ice for 10 min, and centrifuged for 10 min at 4°C. Cells were resuspended in 2 vol of buffer A and disrupted with a Teflon pestle driven at 1500 rpm by a hand-held power drill. Nuclei were collected by centrifugation, the supernatant was removed, and nuclei were resuspended in 5 vol of buffer B (20 mM HEPES [pH 7.5], 0.2 mM EDTA, 1 mM BME, 20% glycerol, 0.5 mM PMSF, and 10 μg/ml of aprotinin, leupeptin, and pepstatin). Nuclei were extracted with 300 mM ammonium sulfate (pH 7.5). Nuclear extracts were centrifuged at 55,000 rpm for 45 min. EMSA were carried out in 20 μl total volume, containing 1–5 μl of nuclear extract at 1–5 μg/ml; 4 μl of 5× buffer A (100 mM HEPES [pH 7.9], 125 mM KCl, 0.5 mM EDTA, 50% glycerol, 10 mM $MgCl_2$); 2 μl of buffer B (10 mM spermidine, 40 mM DTT, 1.2% NP-40, 2 mg/ml BSA); 1 μl of 50 mg/ml double-stranded poly[d(I-C)] and 4 ng of labeled oligonucleotide. Reactions were incubated with the probe at room temperature for 20–40 min, then on ice with 3 μl of specific antibodies for 90 min. Samples were applied on native 6% polyacrilamide gels that were run at room temperature until the xylen-cyanol blue reached 6 cm from the bottom of the gel.

In Situ Detection of Apoptosis and Immunofluorescence

SAOS-2 cells were transfected on cover slides in 24-well dishes at 60% confluence. Twenty-four hours after trasnfection they were treated with doxorubicin (5 μg/ml final concentration) and incubated for additional 12–16 hr. Samples were fixed and permeabilized in buffer A (0.1% Triton X, 150 mM sodium citrate in 1× PBS). After blocking (10% BSA, 20% goat serum in 1× PBS) for 1 hr at 37°C, cells were incubated with the anti-p53 antibody (1:500 [v/v]) for 1.5 hr at 37°C, washed 5 times in buffer A, and incubated with rodhamine conjugated anti-mouse antibody (1:500 dilution [v/v]) for 45 min at 37°C. Detection of apoptotic cells was carried out by using a fluorescein-based TUNEL assay (Boerhinger Mannheim). Samples were analyzed by using a confocal microscope (laser scanning confocal imaging system, MRC-1024, Bio-Rad).

Cell Cycle Analysis

Cells were transfected with the pCMV-IL2R plasmid together with expression vector encoding p300 as described above. Sixteen to 18 hr after transfection, cells were exposed to a total of 6.3 Gy, ^{137}Cs source delivering gamma-rays at a dose rate of 3.46 Gy/min. Four hours after irradiation, nocodazole (SIGMA) was added at a final concentration of 15 μg/ml, followed by an additional incubation of 16–18 hr. Transfected cells were isolated by magnetic affinity cell sorting 30 min after BrdUrd labeling as described previously (Giordano et al., 1991).

Acknowledgments

This work was partially funded by the Lucilla Markey trust to the University of Buffalo. Robert G. Summers and Alan T. Stonebraker are gratefully acknowledged for exceptional assistance with the confocal microscopy. The authors thank Dr. Bruce Howard and Dr. Albert J. Fornace for access to the FACScan and to the gamma-irradiator, respectively. Dr. Ettore Appella, Dr. Richard Eckner, Dr. David Livingston, and Dr. Moshe Oren are acknowledged for providing several vectors employed in this study. The authors thank Dr. Victor J. Hernandez, Dr. Massimo Levrero, Dr. Yoshihiro Nakatani, Dr. Edward G. Niles, Dr. Thomas Melendy, and Dr. Lorenzo Puri for helpful discussions and criticism during the preparation of the manuscript.

Received May 7, 1997; revised June 13, 1997.

References

Angel, P., and Karin, M. (1991). The role of Jun, Fos and the AP1 complex in cell-proliferation and transformation. Biochem. Biophys. Acta *1072*, 129–157.

Arias, J., Alberts, A.S., Brindle, P., Claret, X.S., Smeal, T., Karin, M., Feramisco, J., and Montminy, M. (1994). Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. Nature *370*, 226–229.

Avantaggiati, M.L., Carbone, M., Graessman, A., Nakatani, Y., Howard, B., and Levine, A.S. (1996). The SV40 large T antigen and adenovirus E1a oncoproteins interact with distinct isoforms of the transcriptional coactivator p300. EMBO J. *15*, 2236–2248.

Bannister, A.J., and Kouzarides, T. (1996). The CBP co-activator is a histone acetyltransferase. Nature *384*, 641–643.

Bates, S., and Vousden, K.H. (1996). p53 in signaling checkpoint arrest or apoptosis. Curr. Opin. Genet. Dev. *6*, 12–18.

Borrow, J., Stanton, V.P., Jr., Andersen, J.M., Becher, R., Behm, F.G., Chaganti, R.S., Civin, C.I., Discteche, C., Dube, I., Frichauf, A.M., Horsman, D., Mitelman, F., Volinia, S., Watmore, A.E., and Housman, D.E. (1996). The translocation t(8;16) (p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. Nature Genet. *14*, 33–41.

Chen, K.V., Ueda, K., Pastan, I., and Gottesman, M.M. (1992). Modulation of activity of the promoter of the human MDR1 gene by ras and p53. Science *255*, 459–462.

Deb, S., Jackson, C.T., Subler, M.A., and Martin, D.W. (1992). Modulation of cellular and viral promoters bu mutant human p53 proteins found in human tumors. J. Virol. *66*, 6164–6170.

Eckner, R., Ludlow, J.W., Lill, N.L., Oldread, E., Arany, Z., Modjtahedi, N., De Caprio, J.A., and Livingston, D.M. (1996a). Association of p300 and CBP with simian virus 40 large T antigen. Mol. Cell. Biol. *16*, 3454–3464.

Eckner, R., Yao, T.P., Oldread, E., and Livingston, D.M. (1996b). Interaction and functional collaboration of p300/CBP and bHLH proteins in muscle and B-cell differentiation. Genes Dev. *10*, 2478–2490.

Fan, S., Simth, M.L., Rivet, D.J., Duba, D., Zhan, Q., Kohn, K.W., and Fornace, A.J., Jr. (1995) Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifilline. Cancer Res. *55*, 1649–1654.

Giordano, T., Howard, T., Coleman, J., Sakamoto, K., and Howard, B. (1991). Isolation of a population of transiently transfected quiescent and senescent cells by magnetic affinity cell sorting. Exp. Cell. Res. *192*, 193–197.

Haffner, R., and Oren, M. (1995). Biochemical properties and biological effects of p53. Curr. Opin. Genet. Dev. *5*, 84–90.

Haupt, Y., Rowan, S., Shaulian, E., Vousden, K., and Oren, M. (1995). Induction of apoptosis in HeLa cells by transactivation-deficient p53. Genes Dev. *9*, 2170–2183.

Hinds, P., Finlay, C., and Levine, A.J. (1989). Mutation is required to activate the p53 gene for cooperation with the ras oncogene and transformation. J. Virol. *63*, 739–746.

Janknecht, R., and Hunter, T. (1996a). Transcription. A growing coactivator network. Nature *383*, 22–23.

Janknecht, R., and Hunter, T. (1996b). Versatile molecular glue. Transcriptional control. Curr. Biol. *6*, 951–954.

Jenkins, J.R., Chumakov, P., and Curie, G.A. (1985). The cellular

oncogene p53 can be activated by mutagenesis. Nature *317*, 816–817.

Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., and Glass, C.K. (1996). A CBP integrator complex mediates transcriptional activation and AP1 inhibition by nuclear receptors. Cell *85*, 403–414.

Ko, L.J., and Prives, C. (1996). p53: puzzle and paradigm. Genes Dev. *10*, 1054–1072.

Lee, J.S., See, R.H., Deng, T., and Shiw, Y. (1996). Adenovirus E1a downregulates cJun and JunB-mediated transcription by targeting their coactivator p300. Mol. Cell. Biol. *16*, 4312–4326.

Levine, A.J. (1997). p53, the cellular gatekeeper for growth and division. Cell *88*, 323–331.

Lill, N.L., Tevethia, M.J., Eckner, R., Livingston, D.M., and Modjtahedi, N. (1997). p300 family members associate with the carboxyl terminus of simian virus 40 large tumor antigen. J. Virol. *71*, 129–137.

Michalovitz, D., Halevy, O., and Oren, M. (1990). Conditional inhibition of transformation and cell proliferation by a temperature sensitive mutant of p53. Cell *62*, 671–680.

Miyashita, T., Kraiewsky, S., Krajewska, M., Wang, H.G., Lin, H.K., Liebermann, D.A., Hoffman, B., and Reed, J.C. (1994). Tumor suppressor p53 is a regulator of bcl2 and bax gene expression in vitro and in vivo. Oncogene *9*, 1799–1805.

Moran, E. (1993). DNA tumor virus transfroming proteins and the cell cycle. Curr. Opin. Genet. Dev. *3*, 63–70.

Muraoka, M., Konishi, M., Yanoshita, R., Tanaka, K., Shitara, N., Chong, J.M., Iwama, T., and Miyaki, M. (1996). p300 gene alterations in colorectal and gastric carcinomas. Oncogene *12*, 1565–1569.

Murphy, M., Hunman, A., and Levine, A.J. (1996). Wild-type p53 negatively regulates the expression of a microtubule-associated protein. Genes Dev. *10*, 2971–2980.

Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B., and Nakatani, Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell *87*, 953–959.

Perkins, N.D., Felzien, L.K., Betts, J.C., Leung, K., Beach, D., and Nabel, G.J. (1996). Regulation of NF-κB by cyclin dependent kinases associated with p300 coactivator. Science *275*, 523–527.

Puri, L., Avantaggiati, M.L., Balsano, C., Sang, N., Giordano, A., Graessmann, A., and Levrero, M. (1997). p300 is required for MyoD-dependent cell cycle arrest and muscle-specific gene transcription. EMBO J. *16*, 369–383.

Shen, Y., and Shenk, T. (1994). Relief of p53-mediated transcriptional repression by the adenovirus E1B-19 kDa protein or the cellular Bcl-2 protein. Proc. Natl. Acad. Sci. USA *91*, 8940–8944.

Yaciuk, P., and Moran, E. (1991). Analysis with specific polyclonal antiserum indicates that the E1a-associated 300-kDa product is a stable nuclear phosphoprotein that undergoes cell cycle phase-specific modification. Mol. Cell. Biol. *11*, 5389–5397.

Yang, X.J., Ogryzko, V.V., Nishikawa, J., Howard, B.H., and Nakatani, Y. (1996). A p300/CBP associated factor that competes with the adenoviral oncoprotein E1a. Nature *382*, 319–324.

Yuan, W., Condorelli, G., Caruso, M., Felsani, A., and Giordano, A. (1996). Human p300 protein is a coactivator for the transcription factor Myo D. J. Biol. Chem. *271*, 9009–9013.